Study Title

Fish, Acute Toxicity Test with

PES Vorstufe 2342

Data Requirements / Test Guidelines

EU method C.1 (2008)

OECD TG 203 (1992)

Author:

Dr. Christine Richter

Study completion date:

2011-06-14

Sponsor:

Bayer MaterialScience AG

BMS-IO-ST-PSRA-PRA

51368 Leverkusen

Germany

Testing facility:

CURRENTA GmbH & Co. OHG

Analytik

51368 Leverkusen

Germany

Monitor:

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Laboratory Project Identification

Study No. 2010/0087/11

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CONTENTS

1. GLP DECLARATION

This study was conducted in compliance with the OECD principles of Good Laboratory Practice (1999) and with the Principles of Good Laboratory Practice according to Annex I, German Chemical Law (2008).

Date / Signature

Study Director 2011-06-14 Ch. Richter
(Dr. Christine Richter)

2. ARCHIVING

The original report, the study plan, and all raw data pertaining to this study are stored in the "GLP Archive, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building Q 18, 51368 Leverkusen". A sample of the test item is stored in "GLP-Sample Store, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building DA1, 41538 Dormagen".

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3. QUALITY ASSURANCE STATEMENT

This report was audited by the Quality Assurance Unit CURRENTA Analytik, Quality Management at CURRENTA GmbH & Co. OHG and this statement confirms that the final report reflects the raw data.

The dates of Quality Assurance inspections and audits are given below.

Audits	Dates of QAU inspections	Dates of reports
study plan review	2011-05-04	2011-05-04
inspection of experimental phase	2011-05-09	2011-05-09
inspection of experimental phase	2011-05-12	2011-05-12
final report review (draft)	2011-06-09	2011-06-09
final report review	2011-06-15	2011-06-15

Date / Signature

2011-06-15 A. Senic

(Senic/ ~~Dr. Dörzbach-Lange/ Dr. Neupert~~)

4. STUDY TIME TABLE

Study initiation date:	2011-05-04
Study completion date:	2011-06-14
Start of experimental phase:	2011-05-07
End of experimental phase:	2011-06-01

5. GLP CERTIFICATE

Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz
des Landes Nordrhein-Westfalen

Postanschrift: 40190 Düsseldorf

Aktenzeichen: VI-3- 31.11.65.05

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer Industry Services GmbH & Co OHG
Prüfeinrichtung BIS-SUA-Analytics
D-51368 Leverkusen

(unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV-GLP Nr. 5.3/OECD guidance)
Kategorie 1	category 1
Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	physical-chemical testing
Kategorie 4	category 4
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	environmental toxicity studies on aquatic and terrestrial organisms
Kategorie 5	category 5
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	studies on behaviour in water, soil and air; bioaccumulation
Kategorie 8	category 8
Analytische Prüfungen an biologischen Materialien	analytical and clinical chemistry testing

Datum der Inspektion (Tag.Monat.Jahr)	Date of Inspection (day.month.year)
14. bis 16. September und 26. bis 28. Oktober 2005	on 14 until 16 September and on 26 until 28 October 2005
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.	The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.	Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, den 11. Januar 2006
Im Auftrag

(Prof. Dr. David)

Dienstsiegel/official-seal

Please note: Effective January 1st, 2008 the company name Bayer Industry Services GmbH & Co. OHG was changed to CURRENTA GmbH & Co. OHG

6. SUMMARY

A study was performed to assess the acute toxicity of PES Vorstufe 2342 to *Danio rerio* under static conditions.

The study was conducted in accordance with Council Regulation (EC) No 440/2008, Method C.1 'Acute toxicity for Fish' (2008) which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 203 'Fish, Acute Toxicity Test' (1992).

A group of seven fish of the recommended size was exposed to a limit test concentration of nominally 100 mg/L of PES Vorstufe 2342 dissolved in dilution water. Auxiliaries used to prepare the test media were an ultra turrax and a magnetic stirrer. Undissolved particles of the test item were removed using an aseptic filter.

Observations were made on the number of dead fish and the incidence of sub-lethal effects after 2, 24, 48, 72 and 96 hours of exposure. The following values were determined:

Time [h]	**LL 50 [mg/L]**
2	> 100
24	> 100
48	> 100
72	> 100
96	> 100

No toxic effects against fish were observed at the limit of water solubility.

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established.

The results are expressed in terms of Lethal Loadings (LL). As the test item is a multi constituent and no information about the correlation between molecular weight and the structural formula of the test item are available, a Water Accommodated Fraction (WAF) was used to test effects at a limit concentration of 100 mg/L, and no specific analysis was performed. With the sponsor’s agreement, the content of the test item during the exposure period was verified by DOC determination.

The hardness of the dilution water used was 14.0 °dH (= 250 mg/L $CaCO_3$).

7. EXPERIMENTAL PROCEDURE

The method described in the Council Regulation (EC) No 440/2008, Method C.1 'Acute toxicity for Fish' (2008) which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 203 'Fish, Acute Toxicity Test' (1992) assesses the acute toxic effects (mortality) of various concentrations of a test item to a freshwater fish species.
The purpose of this method was to determine the acute toxic effects at a maximum leathal loading of 100 mg/L.

An acute daphnia and an algae toxicity test preceded the fish test. They provided information about the concentration which was used in the fish test.

The fish test was conducted as static test with the test medium unchanged throughout the duration of the test. As no toxic effects against fish were observed, no statistical analysis was required to determine the LL 50. Additionally any abnormal behaviour or appearance of the fish was reported every 24 hours.

During the test a temperature range of 20 - 24 °C was maintained in the test vessels, with a maximum temperature fluctuation of +/- 1 °C in each individual test. The temperature, the pH and the oxygen values were measured at the beginning of the test and every 24 hours thereafter.

The following validity criteria of the test were met:

The mortality in the controls did not exceed 10 % by the end of the test.

The dissolved oxygen concentration remained above 60 % of the air-saturation value throughout the exposure period.

The pH did not vary by more than 1 unit.

8. MATERIALS AND METHODS

8.1 Sample description

Test item	:	PES Vorstufe 2342
Chemical name	:	Castor Oil, reaction product with Soybean Oil
CAS name	:	--
CAS number	:	--
EC/NLP number	:	919-697-6
Sample provided by	:	Bayer MaterialScience AG
Empirical formula	:	--
Molecular mass	:	--
Batch number	:	LB06603520
Charge	:	--
Sample number	:	1199
Date of receipt	:	2010-04-27
Expiry date	:	2011-09-11
Purity	:	100 % (according to data of the sponsor)
Water solubility	:	0.0058 g/l
Vapour pressure	:	ca. 4 hPa at 20 °C
Stability of test concentration during exposure	:	Examined by chemical analysis (DOC) at 0 and 96 hours.

8.2 Test species

Name	:	Zebra fish (*Danio rerio*)
Source	:	Aqua KlöGer (Germany)
Date of birth	:	--
Date supplied	:	2011-04-19
Acclimatisation	:	Stock held since 2011-04-19 and acclimatised to the test conditions since then.
Temperature	:	20 - 24 °C
Dissolved oxygen	:	> 5 mg/L
Feeding	:	Commercial fish food, daily. Feeding discontinued 24 h prior to test start.
Mortalities during acclimatisation period	:	< 5 %
Medication	:	none
Mean standard Length (n = 14)	:	3.4 cm (S.D. = 0.19 cm)

8.3 Holding and dilution water

Reconstituted water prepared according to the recommendations of ISO 7346. This freshly prepared standard dilution water was used for the maintenance of the test animals under flow-through conditions and for the preparation of stock and test solutions of the test item.
The total hardness of the dilution water, measured at test start, was 14.0 °dH (= 250 mg/L $CaCO_3$).

8.4 Apparatus

Analytical balance

pH meter

Oxygen meter

Incubator

Various glass materials: glass aquaria, glass plates, volumetric flasks, pipettes etc.

8.5 Pre-treatment of test item and preparation of test item concentration

A direct weighing was prepared to produce the only test concentration. 500.2 mg of the test item were added to 5 litres of dilution water, treated with an ultra turrax for 60 sec. at 8000 rpm and was then stirred for 24 h on a magnetic stirrer. Finally undissolved particles of the test item were removed by filtration using an aseptic filter with a pore size of 0.45 + 0.2 µm . The pH was measured to be 7.3. Finally 7 fish were given to the test item concentration and the control.

8.6 Exposure conditions

Test vessels	:	glass aquaria holding 5 L of test media covered by glass plates
Experimental design	:	1 test concentration plus 1 control
		7 fish per test concentration
		no feeding during the exposure period
		static system
Method of initiation	:	fish were placed in prepared media
Loading	:	0.45 g body weight (wet weight) per litre
Photoperiod	:	16 h light: 8 h dark
Temperature	:	22.0 to 22.7 °C
Aeration	:	gentle aeration via narrow glass tubes
Test item concentration	:	100 mg/L
Method of administration	:	direct weighing

Medium renewal	:	none
Duration of exposure	:	96 hours
Criteria of effects	:	The criterion of death used in this study was the absence of response to physical stimulation. In addition to observations on mortality at 2, 24, 48, 72 and 96 hours, type and incidence of sub lethal effects compared with control fish were observed.

8.7 Chemical analysis

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established. With the sponsor's agreement, the content of the test item during the exposure period was verified by DOC determination.

Analytical Standards

Analytical Standard for Determination of Organic Carbon

Potassium hydrogen phthalate, dried at 105 °C for 1 hour, purity > 99.9 %
Potassium hydrogen phthalate (nominal value: 2.125 g) was dissolved in water and made up to the mark in a 1000 mL volumetric flask to prepare a stock solution of 1000 mg Carbon per litre. Defined volumes of the stock solution were diluted with water to obtain standard solutions in the range of 5 to 300 mg/L.

Analytical Standard for Determination of Inorganic Carbon

Sodium carbonate, dried at 285 °C for 1 hour, purity > 99.9 % Sodium hydrogen carbonate, dried for 2 hours over silica gel, purity > 99.9 %
Sodium carbonate (nominal value: 4.415 g) was dissolved in about 500 mL water. Sodium hydrogen carbonate (nominal value: 3.500 g) was added and made up to the mark in a 1000 mL volumetric flask to prepare a stock solution of 1000 mg Carbon per litre. Defined volumes of the stock solution were diluted with water to obtain standard solutions in the range of 15 to 150 mg/L.

Analytical Procedure

Principle

Total Carbon (TC) in water was oxidized to carbon dioxide by combustion. Inorganic Carbon (IC) was measured separately by acidification and purging. Total Organic Carbon (TOC) was calculated by the following equation:

$$TOC = TC - IC$$

As the bioavailable fraction of organic test items is more appropriately reflected by the Dissolved Organic Carbon (DOC), all biological test solutions were initially filtered through a membrane filter of a pore size of 0.45 µm before any further treatment was performed. In case of low DOC values (< 10 mg/L), DOC was measured after removing inorganic carbon by acidification and purging of carbon dioxide. In this case, DOC value was identical with TC.
Carbon dioxide was determined directly by infrared spectrometry.

Calibration

Linear calibration curves were established by analysing organic standard solutions and inorganic carbon solutions of at least three adequate concentrations. Typically, several calibration curves were used in order to cover the whole concentration range needed.

Limit of quantitation

2 mg/L DOC.

Analysis of samples

The biological test solutions were routinely measured on the day of sampling. If this was exceptionally not possible, the samples were stored in a refrigerator at 4 °C until the analysis was carried out. The biological test solutions were analysed in the same way as the calibration samples.

Evaluation of results

Injected samples were quantified by peak areas with reference to the respective calibration curve. The latter was obtained by correlation of peak area of the standard solutions to their corresponding concentration in mg/L. The correlation was performed using a linear function:

$$y = m \cdot x + b$$

y	= peak area of injected sample (counts)
x	= DOC of injected sample (mg carbon per litre)
m	= constant factor, slope of calibration curve
b	= intercept, point of intersection between calibration curve and y-axis

Factor 'Molecular weight / Organic C content': ---

Sampling schedule:

Control	:	at 0 and 96 hours
Test concentration	:	at 0 and 96 hours

8.8 Applied SOPs and methods

00139 V.1	Acute Fish test
2011-0616101-07 D	Hardness of water
2011-0479301-94 D	Determination of metals
2011-0615201-07 D	DOC determination

Deviations: According to the demands of the used guideline the test was performed with 7 fish per concentration

9. RESULTS

Table 1: Control

Abiotic parameters	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
Temperature [°C]	22.1	22.7	22.5	22.1	22.2
Oxygen [mg/L]	8.2	8.1	9.0	8.4	8.8
Oxygen [% saturation]	93	93	103	96	100
pH value	7.3	7.1	7.3	7.2	7.2
Mortality for zebra fish (initial population: 7)	**2 h**	**24 h**	**48 h**	**72 h**	**96 h**
Absolute	0	0	0	0	0
Cumulative	0	0	0	0	0
Cumulative [%]	0	0	0	0	0
Abnormalities *	**2 h**	**24 h**	**48 h**	**72 h**	**96 h**
Abnormal swimming action	0/7	0/7	0/7	0/7	0/7
Abnormal behaviour	0/7	0/7	0/7	0/7	0/7
Chemical analysis	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
DOC mean value [mg/L]	<2	-	-	-	<2

Comments: * Fish with effects / fish alive

Table 2: **100 mg/L**

Abiotic parameters	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
Temperature [°C]	22.0	22.5	22.5	22.5	22.6
Oxygen [mg/L]	9.3	9.0	8.8	8.2	8.7
Oxygen [% saturation]	106	91	100	95	100
pH value	7.4	7.1	7.3	7.2	7.3
Mortality for zebra fish (initial population: 7)	**2 h**	**24 h**	**48 h**	**72 h**	**96 h**
Absolute	0	0	0	0	0
Cumulative	0	0	0	0	0
Cumulative [%]	0	0	0	0	0
Abnormalities *	**2 h**	**24 h**	**48 h**	**72 h**	**96 h**
Abnormal swimming action	0/7	0/7	0/7	0/7	0/7
Abnormal behaviour	0/7	0/7	0/7	0/7	0/7
Chemical analysis	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
DOC mean value [mg/L]	<2				<2

Comments: * Fish with effects / fish alive

An analysis of the mortality data gave the following results:

Time [h]	LL 0 [mg/L]	LL 100 [mg/L]	LL 50 [mg/L]
2	≥ 100	> 100	> 100
24	≥ 100	> 100	> 100
48	≥ 100	> 100	> 100
72	≥ 100	> 100	> 100
96	≥ 100	> 100	> 100

No toxic effects against fish were observed at the limit of water solubility.

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established.

The results are expressed in terms of Lethal Loadings (LL). As the test item is a multi constituent and no information about the correlation between molecular weight and the structural formula of the test item are available, a Water Accommodated Fraction (WAF) was used to test effects at a limit concentration of 100 mg/L, and no specific analysis was performed. With the sponsor's agreement, the content of the test item during the exposure period was verified by DOC determination.

9.1 Comments

Two test fish had a somewhat larger size than the one recommended in the test guideline for zebra fish. This deviation from the recommendation of the guideline is not regarded to be relevant to the results as no behavioural or other abnormalities were observed within the test period.